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PCBs Disturb Differentiation of Normal Human Neural Progenitor Cells: Clue for Involvement of Thyroid Hormone Receptors.

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Abbreviations:

AhR arylhydrocarbon receptor

AhRR AhR repressor

CYP cytochrome P450

GFAP glial fibrillary acidic protein

GST glutathione-S-transferase

NHNP cells normal human neural progenitor cells

NSE neuron specific enolase

PCB polychlorinated biphenyls

RA retinoic acid

RAR retinoic acid receptor

RXR retinoic x receptor

T_3 triiodothyronine

TH thyroid hormone

TR thyroid hormone receptor

UGT UDP glucuronosyl transferase

Outline of section headers:

Abstract

Introduction

Materials and Methods

Results

Discussion

References

Abstract

Polychlorinated biphenyls (PCBs) are ubiquitous environmental chemicals that accumulate over the food chain in adipose tissues. Epidemiological studies have indicated that PCBs influence brain development. Children who are exposed to PCBs during their development suffer from neuropsychologic deficits such as a lower full-scale IQ, reduced visual recognition memory, attention and motor deficits. The mechanisms leading to these effects are not fully understood. It has been speculated that PCBs may affect brain development by interfering with thyroid hormone (TH) signaling. Since most of the data have been gained from animal studies, we established the model of primary normal human neural progenitor cells (NHNP cells) to analyze if PCBs interfere with TH dependent neural differentiation.

NHNP cells differentiate into neurons, astrocytes and oligodendrocytes in culture. They express a variety of drug metabolism enzymes and nuclear receptors. Like triiodo thyronin (T_3), treatment with the mono ortho substituted PCB118 (0.01 – 1 μM) leads to a dose dependent increase of oligodendrocyte formation. This effect was congener specific, since the coplanar PCB126 had no effect. Similar to the T_3 response, the PCB mediated effect on oligodendrocyte formation was blocked by retinoic acid (RA) and thyroid hormone receptor (TR) antagonist NH-3. These results suggest that PCB118 mimics T_3 action via the TH pathway.

Introduction

Polychlorinated biphenyls (PCBs) are anthropogenic industrial chemicals, the production of which was banned in the 1970s because of their presumed carcinogenicity (Chana et al. 2002). However, these chemicals are still present in the food chain; they accumulate in animal and human tissues and remain among the most abundant persistent organic pollutants found in humans (DeKoning and Karmaus 2000; Kim et al. 2004). Depending on their degree of chlorination, they are metabolized to their hydroxy- and/or sulfur containing metabolites (Haraguchi et al. 1997). PCBs can cross the placenta and infants are contaminated via breast milk (DeKoning and Karmaus 2000).

Epidemiological studies have indicated that PCBs influence brain development (rev. by Schantz et al. 2003). Children who are exposed during their development exhibit neuropsychologic deficits such as lower full-scale IQ, reduced visual recognition memory, attention and motor deficits (Ayotte et al. 2003; Darvill et al. 2000; Huisman et al. 1995b; Huisman et al. 1995a; Osius et al. 1999; Walkowiak et al. 2001). Results from studies in rodents supported these findings (Berger et al. 2001; Lilienthal et al. 1990; Roegge et al. 2000; Widholm et al. 2001). PCBs decrease circulating levels of thyroxine (T_4) in animals (Brouwer et al. 1998; Gauger et al. 2004; Meerts et al. 2002). The neuropsychologic findings affected by the exposure to PCBs overlap with those described for maternal thyroid insufficiency (Haddow et al. 1999; Morreale et al. 2000; Pop et al. 1999). Exposure at doses that lower serum TH, however, did not always produce signs of hypothyroidism, e.g. no elevation in TSH (Barter and Klaassen 1992; Kolaja and Klaassen 1998),no lowering of body weight of rat pups (Zoeller et al. 2000) and acceleration of eye opening in rat pups that can also be caused by high levels of thyroid hormone (Goldey et al. 1995).

Epidemiological studies do not uniformly find an association between PCBs and thyroid homeostasis. A negative correlation between circulating levels of thyroid hormone (TH) and

PCB exposure and a positive correlation between the TH regulating hormone thyrotropin (TSH) and PCB exposure was observed (Osius et al. 1999; Schell et al. 2002). Others found no association between PCB exposure and disturbances of the TH pathway. This may be due to combining "high" and "low" exposed groups when comparing them to the reference group. Nevertheless, all observed hormone levels in these epidemiological studies were within the normal range (Hagmar 2003); i.e., accidental exposure to PCBs was not associated with overt hypothyroidism (Nagayama et al. 2001).

Because neither in animals nor in humans is there a clear relationship between PCB exposure, blood TH levels and symptoms of hypothyroidism, several investigators have speculated that PCBs may affect brain development by directly interfering with TH signaling (McKinney and Waller 1998; Porterfield 2000; Porterfield and Hendry 1998). Dowling and Zoeller (2000) showed that RC3/neurogranin expression in the fetal rat brain is controlled by TH from maternal origin. This group also demonstrated that the technical PCB mixture Aroclor1254 regulated the TH dependent genes myelin basic protein and RC3/neurogranin in a TH-like manner in animals (Zoeller et al. 2000). Thus, despite the anti-thyroid effect of PCBs on serum TH, they seem to act in a TH-like way at the cellular level.

Based on these findings, and because no one has critically tested the hypothesis that PCBs can influence developmental events in the human brain we asked: 1) whether PCBs have a TH agonistic/antagonistic effect on human neural development; and 2) which mechanisms are involved? For these purposes we established the model of normal human neural progenitor (NHNP) cells (Brannen and Sugaya 2000), which allow us to study the effect of these environmental chemicals on neural differentiation. Most studies investigating effects of PCBs employ Arochlor which consists of many different PCB congeners. Rather than deal with a heterogeneous group, we chose two different specific PCB congeners: PCB118 is described as a compound with weak dioxin-like activity, while PCB 126 is categorized as a congener

with strong dioxin-like properties (van den Berg et al. 1998). The single congener approach was applied to identify specific PCB involvement in the disturbance of neural differentiation.

Materials and Methods

Chemicals. T_3 (Sigma-Aldrich, Germany) was diluted in ethanol at a concentration of 300 mM. Ortho substituted PCB118 (2,3',4,4',5-pentachlorobiphenyl), coplanar PCB126 (3,3',4,4',5-pentachlorobiphenyl (both Ökometric GmbH, Bayreuth, Germany)), *all trans* retinoic acid (RA (Sigma-Aldrich, Germany)) and the TH antagonist NH-3 (Nguyen et al. 2002) were diluted in DMSO (Sigma-Aldrich, Germany) at a stock concentration of 1.53 mM, 1.,59 mM, 10 mM and 10 mM, respectively. Benzo(a)pyrene (Sigma-Aldrich, Germany) was diluted in tetrahydrofuran (10 mM).

Cell culture and treatment. NHNP cells were purchased from Cambrex BioScience (Belgium) and cultured as neurospheres in NPMM (Neural Progenitor Maintenance Medium, Cambrex BioScience, Belgium) at 37°C with 5% CO_2. Medium was changed every 2-3 days. Upon significant growth (0.7 mm diameter), spheres were chopped with a McIllwaine tissue chopper as described earlier (Svendsen et al. 1998), the resultant cubes formed new spheres within hours and were named P1-P7 with increasing passage after each chopping incident.

For treatment of neurospheres, chemicals were diluted in NPMM with the following final concentrations: T_3 30 nM; PCB 118 and PCB 126 0.01 µM, 0.1 µM and 1 µM; B(a)P 10 µM; RA and NH-3 1 µM each; DMSO 0.065%. Three to ten spheres with a diameter of approximately 0.4 mm each were treated for 7 days prior to plating for differentiation. Treatment was done with these chemicals alone or a cotreatment was performed with PCB118 and NH-3 or RA, respectively, for one week. Differentiation of NHNP cells was initiated by

growth factor withdrawal and plating onto poly-D lysine coated chamber slides (BD Biosciences, Belgium). Neurospheres were plated in a defined medium consisting of DMEM/F12 (3:1) supplemented with N2 (Invitrogen, Karlsruhe). After differentiating for 2 days, cells were fixed in 4% paraformaldehyde for 30 min and stored in PBS at 4°C until immunostaining was performed.

Immunocytochemistry. Fixed slides were washed for 2 x 5 min in PBS. Incubation with the primary antibodies was: a) for the double staining beta(III)tubulin 1:100 and GFAP 1:1000 (both Sigma-Aldrich) in PBS containing 0.3% Triton-X 100 or b) O4 1:15 (Chemicon) in PBS with 10% goat serum for 1h at 37°C followed by 3 10 min washes with PBS. FITC and/or Rhodamine Red coupled secondary antibodies 1:100 each (Jackson Immuno Research, Dianova, Hamburg) were used for detection by incubating 30 min at 37°C followed by 3 10 min washes with PBS. In the 3rd wash, Hoechst 0.1 µg/ml was added for nuclear staining. After brief drying, Vectashield® Mounting Medium (Vector Laboratories, USA) was used to mount the slides before they were covered with cover glass and sealed with nail polish.
Slides were examined with a fluorescent microscope (Olympus) and pictures were taken with a digital camera (colorview XS, Olympus). The number of O4+ oligodendrocytes was determined for each individual sphere by manual counting.

Statistical analysis. The counts were approximately lognormally distributed. Therefore, the geometric mean and the standard deviation of the geometric mean were used. T-test was used after logarithmic transformation of the values. Each treatment was compared to its respective control. The inhibition values were not logarithmically transformed.

RNA preparation and RT-PCR. Total RNA was prepared from 10 to 15 pooled untreated and undifferentiated spheres (passages 0 to 2) using the Absolutely RNA™ Microprep Kit

(Stratagene, The Netherlands). Reverse transcription (RT) and polymerase chain reaction (PCR) were performed as previously described (Döhr et al. 1995). Sequences and annealing temperatures of the used PCR primers are listed in Table 1. Fragments were separated on a 3% agarose gel containing ethidium bromide and visualized under UV light. A 100 bp marker (peqlab, Erlangen) was used to estimate the appropriate sizes of the PCR fragments.

Results

Cultivation and molecular characterization of NHNP cells. Neurospheres were successfully kept in suspension culture over several months. When exceeding the size of 0.7 mm in diameter, they were passaged by chopping into 0.3 mm cubes. This passaging was performed up to 7 times during the lifespan of the NHNP cells. Plating of spheres onto poly-D lysine coated chamber slides under withdrawal of growth factors resulted in quick radial outgrowth and differentiation of the cells (Fig. 1). After immunostaining the differentiated cells were identified as neurons, astrocytes and oligodendrocytes (Fig. 2). Furthermore, neurons seem to form a neuronal network.

For molecular characterization of NHNP cells we performed RT-PCRs of cell type specific genes throughout the first three passages. We could identify typical gene products for the three different cell lineages in undifferentiated neurospheres: neuron specific enolase (NSE) for neurons, glial fibrillary acidic protein (GFAP) for astrocytes (Fig. 3) and proteolipid protein with its splicing variant dm20 (data not shown) for oligodendrocytes. Finding these cell specific markers in undifferentiated cells implies that specific cell fate is determined before plating and differentiation of cells.

To ascertain if NHNP cells are suitable for neurotoxicological studies, we characterized them for their expression of genes playing a role in xenobiotic metabolism. The results obtained from undifferentiated neurospheres are shown in Fig. 3. NHNP cells express the aryl

hydrocarbon Receptor (AhR) and the AhR Repressor (AhRR), which represent central proteins in the regulation of AhR battery genes. Concerning phase 1 enzymes, we could detect gene products for cytochrome P450 (CYP)1A1, CYP1B1 and CYP2D6, whereas CYP2A6, CYP2B6, CYP2C9, CYP2C19 and CYP3A4 were not expressed. With regard to phase 2 enzymes, NHNP cells do express glutathione-*S*-transferase (GST)M1 and GSTT1, but are abundant for UDP-glucuronosyltransferase (UGT)1A6. Hence, NHNP cells have the ability to metabolize xenobiotics.

Our objective was to investigate endocrine disruption of TH homeostasis in NHNP cells, thus we studied the expression of genes coding for thyroid hormone receptors (TR), retinoid acid (RAR) and retinoid x receptors (RXR) which are crucial molecules in hormone signal transduction. Undifferentiated NHNP cells express TRα_1, β_1 and β_2, as well as RARα, and β and RXRα, β and γ. Therefore they represent a suitable cell model for investigating thyroid hormone disruption.

Effects of T_3 and PCBs on NHNP cells. Our initial goal was to investigate the mechanisms leading to disturbance of human brain development in a human *in vitro* model. Because of the suspicion that disruption of thyroid hormone signaling is involved in impairment of intellectual development by PCBs (rev. by Zoeller and Crofton 2000) and because the timing of oligodendrocyte development seems to be dependent on TH (rev. by Konig and Moura, V 2002), we investigated the occurrence of oligodendrocytes during differentiation of NHNP cells. Therefore, undifferentiated neurospheres were treated with 30 nM T_3 for one week. After two additional days of differentiation, we found a significant increase in the number of oligodendrocytes formed compared to the medium controls (Fig. 4). Treating neurospheres with PCB118 for one week also lead to an increase in oligodendrocyte formation, while PCB126 had no effect. It is noteworthy that the solvent DMSO shows some intrinsic effect in this system (Fig. 4). Thus, PCB118 seems to have a TH-like effect in NHNP cells.

Antagonism of T_3 effects with RA and NH-3. To investigate if the TH-like effect of PCB118 is mediated by TH receptors, NHNP cells were co-treated with 30 nM T_3 or 1 μM PCB118 and/or 1 μM RA and/or 1 μM NH-3. After one week, the number of oligodendrocytes in the neurospheres was counted. Both RA and NH-3 treatment prohibited the formation of oligodendrocytes by T_3 and PCB118, while having no intrinsic activity themselves (Fig. 5). These results support the conclusion that PCB118 acts by interfering with the TR complex.

Discussion

It is now generally accepted that developmental exposure to drugs or chemicals can have adverse effects on the structure or function of the nervous system. Identification of such substances resulted mainly from epidemiological data and animal studies. To prevent harm in humans, it is important to develop *in vitro* approaches because in some cases severe species differences can exist (Harry et al. 1998; Tilson 1996). Here, we present the characterization of a human neural *in vitro* model. To demonstrate the toxicological usefulness of this model, we show the effects of two different single PCB congeners on neural development. Although the ability of PCB congeners to induce cytochrome P450 enzymes was intensively studied in rats (Parkinson et al. 1983), AhR-dependent Toxic Equivalency Factors were revised at an expert meeting organized by the WHO (van den Berg et al. 1998). In this publication, PCB118 was ascribed as a compound with weak dioxin-like activity, while PCB 126 was categorized as a congener with strong dioxin-like properties. The present findings demonstrate that an individual PCB congener known to widely contaminate human populations can alter the course of neural differentiation in primary NHNP cells. This effect was restricted to PCB118 with weak dioxin-like activity, and was not observed following treatment with PCB126, a

dioxin-like congener, despite the fact that these cells express the dioxin receptor (AhR). Moreover, the effect of PCB exposure on oligodendrocyte differentiation was similar to the effect of T_3, and could be blocked by the T_3 antagonist NH-3. Therefore, these findings suggest that non dioxin-like PCB congeners like PCB118 may directly interfere with TH signaling in the developing human brain, altering the course of neural differentiation and potentially accounting for the observation that exposure to PCBs is linked to cognitive deficits in the human population.

We are the first to establish a human primary cell model for investigating endocrine disruption in neural development. NHNP cells, which have the ability to differentiate into the 3 major cell types of the human brain – neurons, astrocytes and oligodendrocytes (Fig. 2) - formed the basis of this model. The number of oligodendrocytes was relatively low, with approx. 30 % of the differentiated cells being neurons and approx. 70% appearing as astrocytes (data not shown). Other laboratories reported a distinct distribution pattern of neurons and glia cells in human neurospheres (Buc-Caron 1995; Caldwell et al. 2001; Kanemura et al. 2002; Messina et al. 2003; Piper et al. 2001). These differences may be due to culture conditions, ages of the embryos/fetuses or the brain areas where the cells were prepared from. Nevertheless, the low abundance of oligodendrocytes in NPHH cells provides a very sensitive system to identify agents that induce their differentiation.

Two important features of our *in vitro* model support their use in studies of chemical exposure on neurodevelopment: their xenobiotic metabolic capacity and their TH signal transduction machinery. mRNA analyses reveal that NHNP cells express a variety of phase 1 and phase 2 enzymes (Fig. 3) which indicates that the cell may be capable of xenobiotic metabolism. This is important because the parent PCB congeners may be metabolized before developing toxicity (James 2001). Regarding the expression pattern of phase-1 and phase –2 enzymes no data are available for the developing human brain. In adult brain though, the expression of CYPs differs partially from NHNP cells (Nishimura et al. 2003). We did not identify

CYP2A6 or CYP3A4 expression in NHNP cells, but adult brain exhibits a relatively high abundance of these enzymes compared to CYP1A1 expression. On the other hand, neurospheres expressed CYP1A1, CYP1B1 and CYP2D6. These enzymes are also present in adult brain (Nishimura et al. 2003). Furthermore, NHNP cells express phase 2 enzymes; GSTM1 and GSTT1 were present in NHNP cells and were found in human brain tissue as well (Sherratt et al. 1997). To the contrary, human adult brain, but not NHNP cells expressed UGT1A6 (King et al. 1999). From the abundance of phase 1 and phase 2 enzymes, we consider NHNP cells to be a suitable toxicological model for studying the effects of xenobiotics on the human developing nervous system.

TH and RA are fundamental for brain development (rev. by Bernal et al. 2003; rev. by McCaffery et al. 2003). They exert their actions through nuclear hormone receptors: TR, RAR and RXR. An important premise for investigating endocrine disruption of the thyroid hormone system by PCB is expression of the involved receptors: TRα_1, β_1 and β_2, as well as all RAR and RXR isoforms with exception of RARγ were present in NHNP cells which is in agreement with the distribution of these receptors in adult rodent brains (Zetterstrom et al. 1999). TR mRNA and protein was also detected in human fetal brain (Bernal and Pekonen 1984; Kilby et al. 2000).

In this study, we found that the mono ortho substituted PCB118 as well as TH lead to an increased formation of oligodendrocytes in NHNP cells. The development of oligodendrocytes, which are the myelin producing cells in the central nervous system, is dependent on TH, which aids proliferation and survival of oligodendrocyte pre-progenitor cells (Barres et al. 1994; Ben Hur et al. 1998; Schoonover et al. 2004). The importance of TH for oligodendrocyte formation was further confirmed in hypothyroid animals exhibiting fewer numbers of oligodendrocytes than control animals (Ahlgren et al. 1997).

The observation that PCBs can have an intrinsic TH-like effect has been made earlier: rat pups exposed to Aroclor 1254 opened their eyes at an earlier timepoint, an effect that is

elicited with an excess of T4 (Brosvic et al. 2002; Goldey et al. 1995). In addition, in pregnant animals Aroclor treatment lead to an increased expression of TH dependent genes like RC3/neurogranin and myelin basic protein in fetal brains (Zoeller et al. 2000), although PCB can cause a decrease of serum TH levels (Gauger et al. 2004; Meerts et al. 2002; Morse et al. 1993; Morse et al. 1996). Most studies performed on the effects of PCBs used Aroclor which are technical mixtures of PCBs containing planar and non-planar congeners. Because of the heterogeneity of these mixtures, we decided to apply a single congener approach with two different pentachlorbiphenyls that have weak and strong dioxin-like activities respectively. Our results show for the first time that PCB118 exerts a TH-like effect on a cellular level in primary human cells by increasing the number of oligodendrocytes (Fig. 4).

In order to find out the molecular mechanism of the action of PCB on oligodendrocytes we tested whether the TH-like effect of PCB118 is mediated through the TH receptor complex. Therefore, the experiments were performed in presence of the specific TR antagonist NH-3. NH-3 binds to the ligand binding domain of the TRs, with selectivity for TRβ over TRα, leading to a conformational change of the receptor with release of TR corepressors. But unlike TH, NH-3 prohibits the subsequent recruitment of TR coactivators. Specificity of TRβ inhibition was shown *in vitro* and *in vivo* (Lim et al. 2002; Nguyen et al. 2002). In the presence of NH-3 the formation of oligodendrocytes by TH and PCB118 was blocked (Fig. 5A) which may indicate that the TRβ complex is involved in PCB 118 mediated effects on oligodendrocyte differentiation. Because Gauger et al. (2004) showed that a large variety of PCBs including PCB118 and their metabolites do not competitively bind to TR, we speculate that the TH-like effect of PCB118 on neural differentiation is due to facilitation of coactivator binding.

As another approach to investigate whether PCB118 acts through the TR complex, NHNP cells were cotreated with RA. As shown, RA anticipated oligodendrocyte formation induced by TH or PCB118 treatment (Fig. 5B). RA binds to the RAR receptor, which shares its

heterodimerization partner RXR with several other nuclear receptors including TR (rev. by Rowe 1997). Therefore, it could be suggested that antagonism of TH or PCB118 by RA is caused through competition over RXR. A similar antagonism of TH by RA has been described (Davis and Lazar 1992), and it has been hypothesized that participation of RXR in other activation pathways may modify the cellular response to TH (Sarlieve et al. 2004).

Regarding the metabolic capacity of these progenitor cells, we can not exclude that the observed induction of oligodendrocytes by PCB118 is a result of a PCB metabolites rather than of the parent substance, and this has to be proven by further experiments. However the observed effect is congener specific because PCB126 did not increase oligodendrocytes in NHNP cells. PCB126 is a coplanar biphenyl which activates the AhR, while PCB118 is a mono-ortho substituted and exerts only weak AhR agonist activity (Hestermann et al. 2000). The disability of benzo(a)pyrene, a classical AhR agonist, to induce oligodendrocyte formation in NHNP cells (data not shown) supports the suggestion that the AhR is not involved in the disturbance of neural differentiation.

In summary, we developed a primary human *in vitro* model for investigating endocrine disruption of neural development. We identified the mono ortho substituted PCB118 as a TH disrupter on human neural development, because it induced oligodendrocyte formation in NHNP cells. On the contrary, PCB126 which is a coplanar AhR ligand showed no hormone like activity. The effects seen after PCB118 treatment seem to be mediated through the TR complex, because they can be antagonized by the TR antagonist NH-3 and RA. The precise molecular mechanisms need to be further elucidated.

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Table 1: sequences of oligonucleotides used to perform RT-PCRs with NHNP cells as shown in Fig. 1.

Gene	Sequences	Size (bp)	Annealing temp (°C)	References
beta actin	FW CCCCAGGCACCAGGGCGTGAT	263	60	(Ihm et al. 2002)
	RW GGTCATCTTCTCGCGGTTGGCCTTGGGGT			
NSE	FW CCCACTGATCCTTCCCGATACAT	254	60	(Kukekov et al. 1999)
	RW CCGATCTGGTTGACCTTGAGCA			
GFAP	FW GATCAACTCACCGCCAACAGC	206	60	(Kukekov et al. 1999)
	RW CTCCTCCTCCAGCGACTCAATCT			
PLP/dm20	FW CCATGCCTTCCAGTATGTCATC	354 plp	59	(Kukekov et al. 1999)
	RW GTGGTCCAGGTGTTGAAGTAAATGT	249 dm20		
CYP1A1	FW TAGACACTGATCTGGCTGCAG	146	60	(Omiecinski et al. 1990)
	RW GGGAAGGCTCCATCAGCATC			
CYP1B1	FW AACGTCATGAGTGCCGTGTGT	360	63	(Sutter et al. 1994)
	RW GGCCGGTACGTTCTCCAAATC			
CYP2A6	FW CAGCTGAACACAGAGCAGATGTACA	227	60	(Yengi et al. 2003)
	RW CGCTCCCCGTTGCTGAATA			
CYP2B6	FW CATTCTTCCGGGGATATGGTG	83	60	(Yengi et al. 2003)
	RW CCTCATAGTGGTCACAGAGAATCG			
CYP2C9	FW GAGGAGTTTTCTGGAAGAGGCAT	130	60	(Yengi et al. 2003)
	RW CAAAATTCCGCAGCGTCAT			
CYP2C19	FW GAGGAGTTTTCTGGAAGAGGCC	76	60	(Yengi et al. 2003)
	RW CATTGCTGAAAACGATTCCAAA			
CYP2D6	FW CTTTCTGCGCGAGGTGCT	96	60	(Yengi et al. 2003)
	RW TGGGTCAGGAAAGCCTTTTG			
CYP3A4	FW TCTCATCCCAGACTTGGCCA	85	60	(Yengi et al. 2003)
	RW CATGTGAATGGGTTCCATATAGATAGA			
UGT1A6	FW TCCTGGCTGAGTATTTGGGCC	562	59	(Strassburg et al. 1997)
	RW GTTCGCAAGATTCGATGGTCG			
GSTM1	FW GAACTCCCTGAAAAGCTAAAGCT	132	60	(Ko et al. 2000)
	RW GTTGGGCTCAAATATACGGTGG			
GSTT1	FW TTCCTTACTGGTCCTCACATCTC	262	60	(Ko et al. 2000)
	RW TCCCAGCTCACCGGATCAT			

TRα1	FW CCCTGAAAACCAGCATGTCAG	150	68	(Silva et al. 2002)
	RW TTCTTCTGGATTGTGCGGC			
TRβ1	FW AAGTGCCCAGACCTTCCAAA	150	68	(Silva et al. 2002)
	RW AAAGAAACCCTTGCAGCCTTC			
TRβ2	FW GGGCTGGAGAATGCATGCGTAGACT	239	68	(Gittoes et al. 1997)
	RW ATTCACTGCCCAGGCCTGTTCCATA			
RAR-α	FW ACCCCCTCTACCCCGCATCTACAAG	226	60	(Kimura et al. 2002)
	RW CATGCCCACTTCAAAGCACTTCTGC			
RAR-β	FW ATTCCAGTGCTGACCATCGAGTCC	349	62	(Kimura et al. 2002)
	RW CCTGTTTCTGTGTCATCCATTTCC			
RAR-γ	FW TACCACTATGGGGTCAGC	195	60	(Kimura et al. 2002)
	RW CCGGTCATTTCGCACAGCT			
RXR-α	FW TTCGCTAAGCTCTTGCTC	113	58	(Kimura et al. 2002)
	RW ATAAGGAAGGTGTCAATGGG			
RXR-β	FW GAAGCTCAGGCAAACACTAC	111	58	(Kimura et al. 2002)
	RW TGCAGTCTTTGTTGTCCC			
RXR-γ	FW GCAGTTCAGAGGACATCAAGCC	352	62	(Kimura et al. 2002)
	RW GCCTCACTCTCAGCTCGCTCTC			
				Accession number/
				Position in sequence
AhR	FW TGGTCTCCCCCAGACAGTAG	132	60	BC070080/
	RW TTCATTGCCAGAAAACCAGA			1113-1244
AhRR	FW CAGTTACCTCCGGGTGAAGA	161	60	NM_020731/
	RW CCAGAGCAAAGCCATTAAGA			269-429

Abbreviations: AhR: arylhydrocarbon receptor; AhRR: AhR repressor; CYP: cytochrome P450; GFAP: glial fibrillary acidic protein; GST: glutathion-S-transferase; NSE: neuron specific enolase; RAR: retinoic acid receptor; RXR: retinoic x receptor; UGT: UDP glucuronosyl transferase; TR: thyroid hormone receptor.

Fig. 1: Neurosphere plated on poly-D lysine coated cover slides. Differentiation and radial outgrowth of cells out of the sphere after 4 days in culture. Phase contrast image. Scale bar represents 200 µm.

Fig. 2: Immunocytochemical staining of differentiated NHNP cells. A) beta(III)tubulin$^+$ neurons (green), GFAP$^+$ astrocytes (red), nuclei stained with Hoechst. Scale bar represents 20 µm. B) O4$^+$ oligodendrocyte. Scale bar represents 100 µm.

Fig. 3: Expression patterns (RT-PCR) of different drug metabolizing enzymes (CYPs, GSTs, UGT), neural markers (NSE, GFAP) and nuclear receptors (TRs, RARs, RXRs) during passaging of undifferentiated NHNP cells (P0 – P2). Reverse transcription (RT) and polymerase chain reaction (PCR) were performed as previously described (Döhr et al. 1995). Respective primer sequences are given in table 1. (note: The unspecific bands in some samples may be caused by the high cycle numbers (40) needed for detection of specific gene products due to the small amount of RNA obtained from each sample.)

Fig. 4: Induction of O4+ oligodendrocytes/sphere by T_3 or PCB118. Pictures show typical results of treatments (scale bars equal 100 µm). Neurospheres were treated with T_3 or PCBs as described in Materials and Methods. Statistical significance calculated with the t-test (* = $p<0.05$; ** = $p<0.01$). Variations indicate standard deviations of the mean. Graphs (geometric means) represent typical representatives of three independent experiments.

Fig. 5: Antagonism of T_3 or PCB118 induced oligodendrocyte formation by A) NH-3 and B) RA. Treatments are described in Materials and Methods. Inhibitions are

shown as percent of T_3 or PCB118 control respectively. Graphs represent typical representatives of three independent experiments.

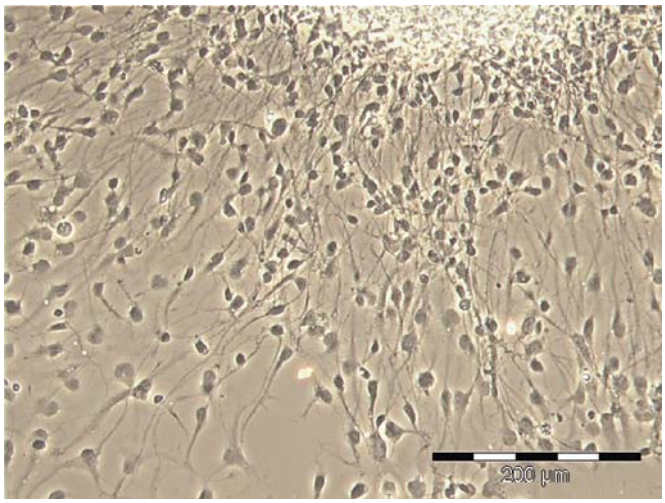


Fig. 1:

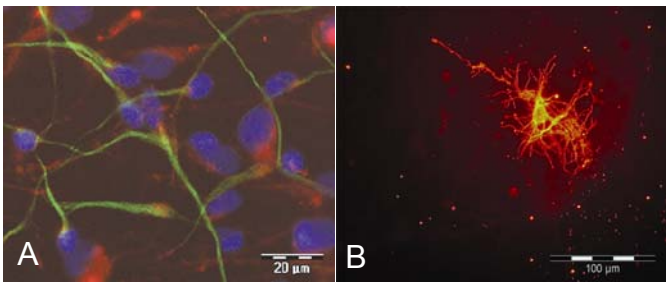


Fig. 2:

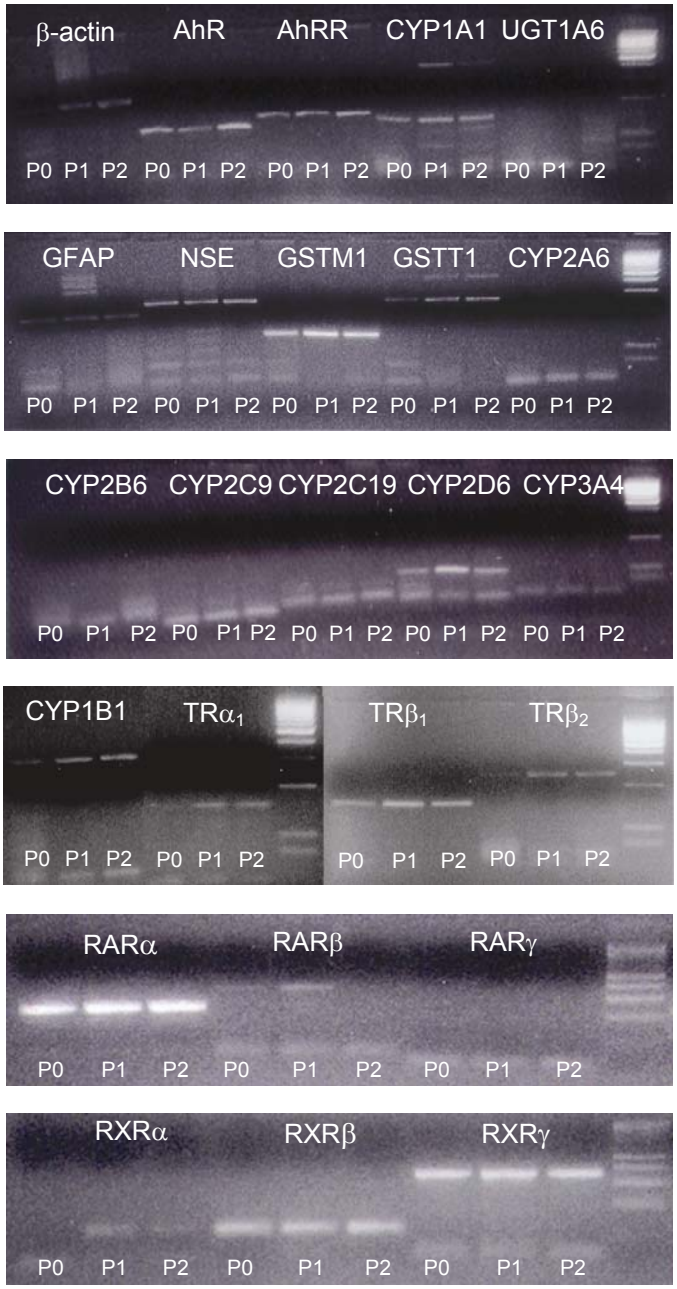

β-actin
AhR
AhRR
CYP1A1
UGT1A6
P0 P1 P2 P0 P1 P2 P0 P1 P2 P0 P1 P2 P0 P1 P2
GFAP
NSE
GSTM1
GSTT1
CYP2A6
P0 P1 P2 P0 P1 P2 P0 P1 P2 P0 P1 P2 P0 P1 P2
CYP2B6
CYP2C9
CYP2C19
CYP2D6
CYP3A4
P0 P1 P2 P0 P1 P2 P0 P1 P2 P0 P1 P2 P0 P1 P2
CYP1B1
TRα1
TRβ1
TRβ2
P0 P1 P2 P0 P1 P2
P0 P1 P2 P0 P1 P2
RARα
RARβ
RARγ
P0 P1 P2 P0 P1 P2 P0 P1 P2
RXRα
RXRβ
RXRγ
P0 P1 P2 P0 P1 P2 P0 P1 P2

Fig. 3:

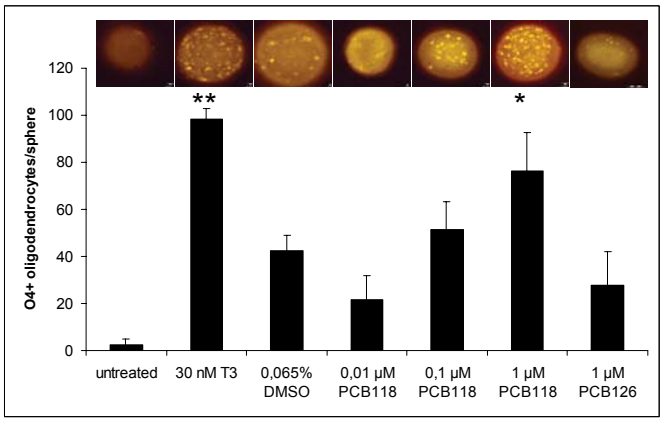
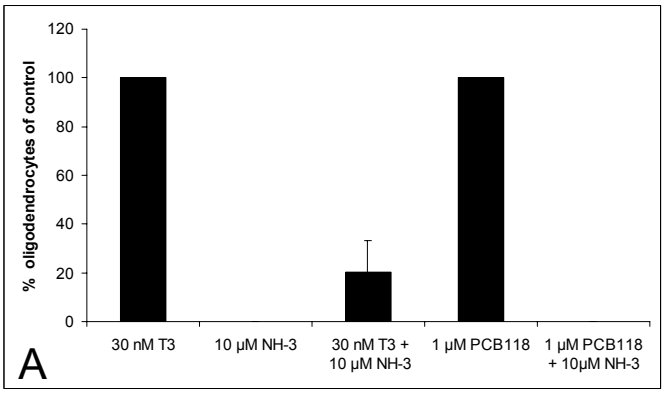

O4+ oligodendrocytes/sphere
120
100
80
60
40
20
0
**
*
untreated
30 nM T3
0,065% DMSO
0,01 µM PCB118
0,1 µM PCB118
1 µM PCB118
1 µM PCB126

Fig.4


% oligodendrocytes of control
120
100
80
60
40
20
0
30 nM T3
10 µM NH-3
30 nM T3 + 10 µM NH-3
1 µM PCB118
1 µM PCB118 + 10µM NH-3
A

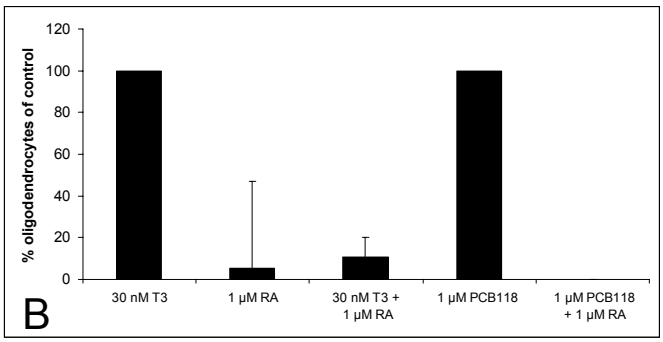

% oligodendrocytes of control
120
100
80
60
40
20
0
30 nM T3
1 µM RA
30 nM T3 + 1 µM RA
1 µM PCB118
1 µM PCB118 + 1 µM RA
B

Fig. 5 A) & B)